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On Some Pitfalls in Developing an Adequate Genetic Hypothesis[1]

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INTRODUCTION

IT WOULD be redundant in this company to extol the advantages of membership in the American Society of Human Genetics. There is, however, one advantage which did not occur to me until several months ago, an advantage which I dare say has not occurred to most of you. This is in connection with the Presidential Address. Many societies are most restrictive in this matter. The president of such a society, who if his society is at all active already has problems enough, is required in the space of forty minutes to develop some broad philosophical topic, or to review succinctly his lifetime of endeavor in his chosen field. Our Society, by what I am sure is careful design, has adopted an entirely different pattern, one which some in this audience will in turn take advantage of. Apparently the only requirement for our Presidential Address is that the speaker talk about some subject close to his heart at the moment. This has already resulted in a remarkable diversity in the Presidential Addresses presented before this Society. Tonight I expect to strike off in still another direction.

By way of introduction to my topic, I should like to draw attention once more to the truly sobering breadth of our field of interest, and the resultant heterogeneity of background among our membership. Everyone likes to feel that his particular interest in science comes closest to being the "key" discipline. In point of fact, there is of course no key discipline in our complexly integrated science today. Yet I submit that there is no aspect of human biology with more ramifications than the study of human heredity. Philosophy, sociology, psychology, anthropology, medicine—all have seen their conceptual framework profoundly affected in recent years by developments within the area represented by our Society. However, this very breadth creates for us serious methodological problems. Human genetics has and must continue to attract the interest of many not primarily trained as geneticists. And, in reverse, trained geneticists may be asked to deal with traits, an appreciation of which calls for an understanding of all the complexities of modern medicine or psychology. It is axiomatic, then, that many of us in our research will frequently be concerned with areas just at the fringe of our knowledge. Intimately familiar with the do's and don't's of investigation in a particular area, our problem of the moment

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may lead us into strange fields. Tonight I would like to discuss with you some of the Scyllas and Charybdises which lie in wait for us as we seek to extend our knowledge of inheritance in man.

Let me at the outset make it clear that in presuming to address you concerning some pitfalls to be avoided, I speak, not from some high vantage point, but as one who has already personally explored a number of these pitfalls, and not from the outside looking in, but from the inside trying to climb out. Little that I have to say is new, but this will scarcely come as a disappointment to any of you, since new ideas in science are, after all, extremely rare and practically never encountered in presidential addresses. In a sense, this presentation may be considered a continuation of the discussion on problems and methods in human genetics sponsored last October by the Morphology and Genetics Study Section, National Institutes of Health, U. S. Public Health Service and the American Cancer Society.

Although there are many matters which could be discussed under the ambiguous title of this address, time permits us to take a hurried look at only four of the problems which the contemporary genetic literature would suggest commonly arise in attempts to develop an adequate genetic hypothesis. Incidentally, these problems are not unique to human genetics but perhaps are more obvious because of certain limitations on the extent to which we can manipulate our material.

1. HETEROGENEITY OF MATERIAL

People and opportunities being what they are, something less than complete freedom of choice plays a hand in the problems which each of us selects for investigation. Still and away, however, we all do enjoy a certain latitude in the kinds of questions with which we come to grips. If one is interested—as most of us are—in developing a satisfactory, unique, and consistent formal explanation for the role of genetic factors in the etiology of a particular trait, then in the present state of our knowledge one is well advised to exercise care in the selection of the trait for study. The more homogeneous the material, the greater the probability of a successful genetic analysis. How does one recognize homogeneity? This is perhaps best answered in a negative fashion. If one assembles cases of a somewhat loosely defined disease or trait of variable age of onset, concerning whose presence or absence competent authorities may disagree, and for which the basis is poorly understood, one is not likely to find oneself with a genetically homogeneous collection of material. Thus, such traits in man as musical ability, superior intelligence, hypertension, idiopathic rotoscoliosis of the spine, or neurasthenia can scarcely be expected to fit into any simple genetic schema. Let me make it clear that I am not suggesting that geneticists should display no interest in such traits, but rather that in approaching them there be a clear recognition of the methodological implications of their probable mixed nature.

Even with the most careful selection, one may still encounter a certain lack of genetic homogeneity in one's material. But while it may not be feasible to eliminate all heterogeneity, one so oriented can at least strive to reduce the picture to the point where genetic analysis leads to the recognition of several biologically meaningful subdivisions of the material at hand. In other words, once one has reduced the

heterogeneity to a certain point, the genetic approach may make it possible to complete the resolution. Two specific examples will help to make my point. The first concerns gargoylism. Herndon, Goodman, and David, in a paper which will appear in an early number of the American Journal of Human Genetics, and from which I am kindly permitted to quote, describe this variably expressed syndrome as follows: "Clinically the affected children present a grotesque appearance which is likened to that of the gargoyles seen on Gothic cathedrals, from which the term "gargoylism" is derived. There is moderate disproportionate dwarfism, the head being large with prominent forehead and plump face. The skull is often scaphocephalic or oxycephalic and hypertelorism is frequent. Kyphosis is usual and extension of the joints is often limited, frequently with a claw-like appearance of the broad hands. The liver and spleen are enlarged, and the abdomen protuberant. Mental deficiency is common and may vary from moron to imbecile levels. Corneal clouding, deafness, hypertrichosis, inguinal or umbilical hernia and recurrent otitis media are frequently described. Cardiovascular disease is often seen.... Radiologic examination discloses widespread skeletal abnormalities, with thickening of the skull, vertebral deformities and wide thick ribs. The bones of the extremities are short and thick with dense structure, and the proximal epiphyses of the humerus and femur are frequently deformed." The basic pathology is a metabolic disturbance, but whether this is of lipid or carbohydrate metabolism remains a moot point.

The familial nature of gargoylism was apparent from the early case reports, several authors mentioning recessive heredity as a possible explanation (e.g., Cockayne, 1936). In 1946 Njå described a pedigree typical of sex-linked heredity, and on the basis of this pedigree plus a survey of the literature, which revealed an excess of affected males over females, suggested the possibility of two genetic types of the disease, one due to autosomal recessive heredity and the other to sex-linked recessive heredity. Herndon, Goodman, and David have now compiled all the case reports they could locate in the literature, finding a very significant excess of males over females (167 ♂♂:79 ♀♀), with some pedigrees published since Njå's also almost certainly illustrating sex-linked recessive heredity. On the assumption that this excess of affected males is due to a mixture of two genetic types of disease, they calculate by a maximum likelihood approach that in approximately 65 per cent of the sibships containing one or more affected persons the disease is due to autosomal recessive heredity, and in the remaining 35 per cent to sex-linked recessive heredity.

Njå noted that in affected members of his pedigree, as well as in several other pedigrees in which only males were affected, the clouding of the cornea so frequently seen in this disease was lacking, and raised the possibility that the sex-linked variety differed phenotypically from the autosomal variety in this respect. Herndon et al. have now divided the accumulated material into three groups, group 1 consisting of all cases observed in sibships containing at least one affected female, and presumably due to autosomal heredity, group 3 consisting of 21 males observed in 7 families wherein the distribution was very suggestive of sex-linked heredity, and group 2 consisting of cases observed in sibships containing only affected males, with presumably both genetic types represented. The cases were then tabulated with respect to certain clinical characteristics of the disease. The results are shown in Table 1.

TABLE 1. THE OCCURENCE OF CERTAIN PHYSICAL FINDINGS IN GARGOYLISM IN RELATION TO THE PROBABLE MODE OF INHERITANCE OF THE DISEASE (AFTER HERNDON, GOODMAN, AND DAVID, IN PRESS). FURTHER EXPLANATION IN TEXT

Clinical Signs	Group I, Autosomal Recessive, 96 Cases		Group II, Mixed, 129 Cases		Group III, Sex-linked, 21 Cases	
	Number	%	Number	%	Number	%
1. Corneal clouding	78	81.3	64	49.6	0	0
2. Dwarfing	70	72.9	60	46.5	7	33.3
3. Deafness	5	5.2	22	17.1	9	42.9
4. Limited joint extension	86	89.6	115	89.2	14	66.7
5. Typical facies	83	86.5	104	80.6	18	85.7
6. Cranial deformity	81	84.4	122	94.6	20	95.2
7. Hepatosplenomegaly	70	72.9	93	72.1	17	80.9
8. Mental defect	68	70.8	97	75.2	11	52.4
9. Spinal deformity	66	68.8	99	76.7	6	28.6
10. Hernia	44	45.8	73	56.6	11	52.4
11. Short neck	44	45.8	56	43.4	10	47.6
12. Otitis media	32	33.3	51	39.5	8	38.1
13. Hypertrichosis	28	29.2	35	27.1	5	23.8
14. Enlarged sella turcica	21	21.9	45	34.9	4	19.1

Groups I, II and III show graded significant differences at the 1% level with respect to items 1, 2 and 3, but not with respect to items 4 through 14.

Corneal clouding, as Njå suggested, emerges as characteristic of the autosomally inherited type; in addition, deafness is only seldom seen in this group but encountered in 43 per cent of the presumably sex-linked group. Thus, the application of genetic techniques serves to sort out of a variably expressed syndrome several somewhat less variable subtypes, i.e., an apparent wide range of variability may be shown in some instances to be due at least in part to genetic heterogeneity.

Our own experience with sickle cell disease provides another illustration of how, where the heterogeneity of the data is not excessive, the genetic approach may actually help unravel that heterogeneity. The marked sickling tendency of the erythrocytes of certain persons, resulting in sickle cell disease, due as it is to the presence of an abnormal hemoglobin, would at first glance appear to be in all likelihood highly specific, genetically speaking, and such was our thought when we initiated our studies of this disease. But it now develops that although the bulk of sickle cell disease apparently does have a uniform genetic basis, being due to homozygosity for a particular gene, there are several additional, much less common, varieties, due to the combination of a single sickle cell gene with the genes responsible for thalassemia, hemoglobin C, or hemoglobin D. At this point the genetic approach makes a major contribution towards clarifying certain ambiguities in the sickle cell story. Recognition that a single sickle cell gene in combination with a thalassemia gene may produce sickle cell disease helps explain the observation that most of the reported cases of sickle cell disease in nominal Caucasians have occurred in individuals of Italian or Greek derivation, since the thalassemia gene has a relatively high frequency in such persons. Likewise, the recognition that the cases of sickle cell disease due to simultaneous heterozygosity for the sickle cell and hemoglobin C genes tend to be

mild helps explain the existence of apparent "intermediates" between the sickle cell trait and classical sickle cell disease (cf. Neel, 1952).

Even with the strictest possible adherence to a set of rigidly defined criteria, we may still encounter heterogeneity in our material. For instance, at last count there were known approximately a dozen different mutants of the shaker-waltzer variety in the house mouse, all inherited as if due to a single autosomal dominant or recessive gene (Grüneberg, 1952). Although some of these mutant strains are distinguished by associated morphological defects, such as syndactylism or coat color changes, and others by the precise behavior pattern of the animals and the course of the disease, there remains a number of strains distinguishable from one another only by genetic tests. In theory, in man a comparable situation could be analyzed in terms of the linkage relationships of the traits—but, as I have pointed out elsewhere (Neel, 1949), the difficulties in man of defining linkage relationships in single pedigrees are very considerable.

An even more striking example of genetic heterogeneity underlying phenotypic similarity is provided by the Tailless-Fused-Kinky mutants of the mouse. These rather similar phenotypes are due to mutation at three or more different loci, all contained within a chromosome segment having a maximum length of about eight cross-over units (Dunn and Caspari, 1945). One of these loci, Tailless, is apparently quite mutable, with some of the "mutations" suppressing crossing-over in immediately adjacent regions, this suggesting a chromosomal rearrangement (Dunn and Gluecksohn-Waelsch, 1953). Even the most enthusiastic student of human genetics must admit the inherent improbability of ever being able to analyze such a situation in man, and the consequent danger that our material is genetically heterogeneous.

Much of modern day genetic research, especially in the fields of psychology and medicine, is team research. This is sometimes a polite way of saying that someone with a collection of data suggesting genetic factors may be at work looks about for some genetic help. Who, now, examines the data from the standpoint of homogeneity? Let me register a strong vote for the geneticist. But, the latter may say, I really don't know that much about the condition in question. To that it can be replied that unless you have a good understanding of the traits involved, how do you decide the gauge at which to run your analysis? After all, one of the first rules of scientific procedure is not to carry third decimal places on data collected by rule of thumb.

It is perhaps relevant at this point to take cognizance of the fact that recently several distinguished scholars in our field have suggested that we may be approaching "the end of what can be accomplished by what we call the traditional, atomistic approach of working with . . . single gene substitutions" (Snyder, 1954; see also Dobzhansky and Wallace, 1954). I am of the opinion that this is the beginning rather than the end, that we are only now in a position to carry out many really basic studies on the physiology and population dynamics of these single genes, and that it would be premature to divert a large portion of our energies into studies of complex genetic situations before we have even begun to appreciate the lessons to be learned from intensive studies of single gene effects, lessons which by extrapolation may be of great value in approaching the more complex. Recent developments in

the field of hematological and serological genetics supply a number of reasons for that judgment (review in Neel, 1954). This point of view implies a continued apparent preoccupation with so-called pathological traits, a preoccupation which some might term undesirable, but it was just such a preoccupation as this that carried Drosophila genetics forward so fast.

In summary, then, it is suggested that there is no faster way to dull the edge of the beautiful mathematical tools which we now possess than to attempt to use these tools in inappropriate situations. A vast amount of work remains to be done on clearly defined traits involving "simple" one- and two-gene situations. From such work should come some of the insight we need for a successful attack on more complicated problems. In the meantime, we minimize the probability of future embarrassment—not to say actual opprobrium—by going no further than a statement as to heritability for many of the more complex traits. The conflict of opinion between such able investigators as Harris (1950) and Steinberg and Wilder (1952) on the genetics of diabetes mellitus, or Kallmann (1953) and Böök (1953) on the genetics of schizophrenia, probably arises primarily from the nature of their material; this conflict will persist until the data can be simplified.

2. PENETRANCE AND THE IMPROPER USE OF SECONDARY HYPOTHESES

The second point to be considered involves the improper use of secondary hypotheses. To no small extent, the strong intellectual appeal which the study of genetics holds for many of us stems from the opportunity to pursue a more precise and mathematical line of reasoning than is granted to most of our colleagues in biology. When studying the genetics of a particular trait, we commonly systematically test our data for agreement with one or several not improbable hypotheses. Recent years have witnessed an increasingly clear recognition of the extent to which such factors as a low level of ascertainment of the trait in the population, uncertainty of medical diagnosis, and a variable age of onset may distort fundamental ratios. These are now matters of common knowledge, which need scarcely be labored with an audience of this background.

There is, however, one problem which arises in the mathematical analysis of genetic data at which we shall take a rather critical look. Let us assume that we have carefully analyzed a set of data for agreement with monogenic or digenic heredity and we find, as is not too uncommonly the case with respect to many traits whose precise pathological basis is unclear, that there is a deficiency between observed and expected affected on each of several simple genetic hypotheses. At this point it is not at all uncommon to see the supplementary hypothesis of "incomplete penetrance" introduced. Thus, in recent years the familial pattern of such diseases as essential hypertension, bronchial asthma, paralysis agitans, rheumatoid arthritis, goiter, paralytic poliomyelitis, neurocirculatory asthenia, diabetes mellitus, and schizophrenia has been suggested to be consistent with monogenic heredity with incomplete penetrance. While some of these suggestions may be correct, others are almost certainly in error. Most geneticists reading the original papers will realize that while this is in one sense the simplest hypothesis, there are in each case several other quite tenable explanations. Unfortunately, the non-geneticist who peruses the

same paper scarcely can be expected to have acquired the background necessary to such judgments, and the sanctity of the printed word being what it is, the statement that such and such a disease is due to a single gene of incomplete penetrance soon becomes well seeded throughout the literature.

Under certain circumstances, as, for instance, in the case of the gene responsible for retinoblastoma, one can speak with considerable assurance of incomplete penetrance, amounting in this particular case to about 90 per cent. But as one descends the scale of penetrance, one arrives at a point, which we may arbitrarily place in the neighborhood of 60 to 70 per cent, where the extravagant use of the concept of incomplete penetrance, particularly in connection with the study of relatively common traits, creates in the end more problems than it solves. A scientifically productive hypothesis is one which suggests further manipulations of the data. The concept of incomplete penetrance seldom does so, since not only can almost any set of data be explained in terms of one gene and a "penetrance factor," but the concept so readily leads to a type of circular reasoning. Thus we say "the data are consistent with heredity due to a dominant gene with 70 per cent penetrance, therefore the gene probably has 70 per cent penetrance," and stop the analysis at this point. But as Stecher, Hersh, Solomon, and Wolpaw (1953) have recently demonstrated in a paper which illustrates the extremes to which the concept of penetrance can be carried, given a familial distribution of disease explicable as due to the effects of a dominant gene of incomplete penetrance, this situation can also often be "explained" by the postulate of dependence upon a recessive gene with a different degree of penetrance. Furthermore, the pedigree distribution of a trait due to the interaction of genes located at two or more loci may be very similar to the distribution of a trait due to an incompletely penetrant gene. On a priori grounds it is difficult to say that one of these possible explanations of a collection of data is more probable than the other, but distinguishing between the two may be mathematically impossible.

Where one is studying a trait in whose etiology genetic factors are important, and which has a variable age of onset, it is clear that one is justified in speaking of non-penetrance in individuals who have the necessary genetic background but are below the age of onset. Under these circumstances the term "non-penetrant" means only that we are unable to recognize any effect of the genetic background at that time. In this respect, it is like the term "recessive," which describes what the geneticist observes rather than any fundamental property of the gene. Continuing advances in our ability to recognize carrier states may increasingly relieve us from the necessity of falling back on the concept of non-penetrance. A case in point is supplied by recent work of Fajans and Conn (in press) on the prediction of susceptibility to diabetes mellitus. Certain individuals with a normal glucose tolerance curve develop an abnormal curve following small doses of cortisone. Among the siblings of diabetics (excluding, of course, those with manifest or occult diabetes), roughly a quarter show such abnormal responses, whereas only a few per cent of "normal" controls respond in this fashion. It is assumed that individuals exhibiting such abnormal responses have an increased risk of developing diabetes at some later date. We have been collaborating with Dr. Fajans and Dr. Conn on the genetic implications of this discovery, and find that when the familial pattern of both diabetes mellitus

and an abnormal response to cortisone is considered, the analytic problems involved in the study of heredity in diabetes may be considerably simplified.

It can confidently be anticipated that the future holds many similar discoveries with reference to genetically conditioned diseases, discoveries which it is to be hoped will minimize the need for the deus ex machina of non-penetrance. In the meantime, perhaps it is desirable in situations such as enumerated previously to speak of heritability in the broad sense rather than to attempt, prematurely, to specify a precise genetic mechanism which has a high probability of confusing the literature and haunting the author.

3. BIAS IN THE SELECTION OF CONTROLS

Thirdly, the matter of the selection and treatment of control material should be mentioned. Once the geneticist departs from the well blazed trail of one- and two-gene heredity and finds himself on the footpaths and backroads of "heritability" and "constitutional factors," the need for adequate controls asserts itself. The less clear-cut the genetics of a situation, the greater the need for adequate controls. In recent years we have seen several striking demonstrations of the manner in which the selection of controls alters the interpretation of one's findings. Time permits the discussion of only one example. In the past decade a number of very substantial studies on heredity in breast cancer have been carried out. Jacobsen (1946) compared the frequency of breast cancer in the relatives of 200 patients with this disease with its occurrence in the relatives of 200 controls with approximately the same age and, presumably, socio-economic distribution. A portion of his findings is reproduced in Table 2. The cancer percentages given in the table are the sum of the mortality from this cause among the deceased relatives in the designated category plus the morbidity among the living relatives in the same category. In very rough terms there appeared to be approximately ten times the amount of cancer in the relatives of the cancer probands than in the relatives of the controls. This is a striking difference. However, Busk (1948) pointed out that by comparison with official Danish mortality and morbidity statistics, there was a very significant deficiency of persons

TABLE 2. THE FREQUENCY OF CANCER OF THE BREAST IN (A) THE FEMALE RELATIVES OF 200 PATIENTS WITH CANCER OF THE BREAST (JACOBSEN), (B) THE FEMALE RELATIVES OF 200 CONTROL PATIENTS (JACOBSEN), AND (C) IN A HYPOTHETICAL CONTROL POPULATION BASED ON DANISH MORTALITY AND MORBIDITY DATA FOR BREAST CANCER (BUSK)

Relationship	Breast Cancer Relatives			Control Relatives			Hypothetical Control		
	Number	Affected	Per cent affected	Number	Affected	Per cent affected	Number	Affected	Per cent affected
Mother	200	21	10.5	200	2	1.0	200	4	2.0
Sister	381	13	3.4	433	2	0.5	433	2	0.5
Maternal grandmother	183	4	2.2	172	—	—	172	4	2.3
Paternal grandmother	157	4	2.5	162	2	1.2	162	4	2.5
Maternal aunt	316	17	5.4	312	—	—	312	5	1.6
Paternal aunt	224	12	5.4	223	2	0.9	233	4	1.7
Total	1461	71	4.9	1502	8	0.5	1502	23	1.5

TABLE 3. A COMPARISON OF THE OBSERVED AND EXPECTED FREQUENCY OF DEATH DUE TO MAMMARY AND NON-MAMMARY CANCER IN THE CLOSE RELATIVES OF WOMEN DYING BECAUSE OF MAMMARY CANCER (AFTER PENROSE, MACKENZIE, AND KARN, 1948)

Relative	Total No. of Deaths	A. Cancer Other Than Mammary		B. Mammary	
		Obs.	Exp.	Obs.	Exp.
Mother	406	51	49.23	25	11.12
Sister	307	19	25.23	23	6.97
Daughter	30	0	0.41	0	0.12
Father	420	41	52.32	0	0.10
Brother	455	29	28.14	1	0.05
Son	50	2	0.81	0	0.00
Total	1668	142	156.14	49	18.36
χ^2 (Obs.-Exp.)^2/Exp		1.3		51.1	

with breast cancer among the controls, raising the strong suspicion that either the "controls" were non-representative of the general population or unfamiliar with the causes of death within their families. When Busk (1948) reanalyzed Jacobsen's data, using Danish vital statistics as control figures, the apparent increase in breast cancer among the mothers of such patients was reduced from ten-fold to three-fold.

Penrose, Mackenzie, and Karn (1948) investigated the frequency of cancer of the breast among the deceased relatives of 510 women with cancer of the breast. As a standard of comparison, they calculated the amount of cancer of the breast to be expected, on the basis of the official death certificates from England and Wales. The findings are shown in Table 3. There is good agreement between this study and Jacobsen's study as corrected by Busk. However, while this approach avoids the problems inherent in assembling controls—problems which have been eloquently enumerated by Macklin (1954; see also Dorn, 1954)—it assumes that the population studied is a miniature of the national population. While this assumption may be safe for areas with a relatively homogeneous population, the assumption would be unsafe in this country except for small and select areas.

Here in this country a number of important studies on familial factors in breast cancer have been carried out in recent years. Much of this material is still unpublished. Accordingly, in the course of preparing this paper, I wrote the principal architects of four of the studies—Dr. Madge Macklin, Dr. D. P. Murphy, Dr. C. P. Oliver, and Dr. Sheldon Reed—concerning the current status of their analyses. The response was most generous; I am extremely grateful to these investigators. In going over the results, one is impressed by two facts:

1) There is in general substantial agreement between these four studies, as well as the two already discussed, in indicating a two- to three-fold increase in the frequency of breast cancer among the mothers and sisters of breast cancer patients.

2) Certain real differences do appear to exist between the findings of the various studies—most notably between the results of Dr. Murphy and Dr. Macklin. I have no desire to inject myself gratuitously into a discussion of the cause of these differ-

ences, a discussion which has already enlivened several sessions of this Society. However, there are immediately apparent three possible explanations for the discrepancies between the various studies, namely, biological differences between the populations involved, procedural differences in the investigations, and differences in the statistical approach to the data. With respect to the latter, one of the basic issues involves the propriety of combining morbidity and mortality data into a single figure, when the various studies may well differ both as regards the relative contribution of the two types of data and the age distribution of the individuals contributing to the data. Without expressing an opinion, I can perhaps voice the hope that the methods and data are presented in such a way that interested persons can judge for themselves the possible influence of various statistical treatments.

The point at issue in the present context is that the importance one ascribes to familial factors in the etiology of carcinoma of the breast is strongly influenced by the selection and treatment of controls. When the four largely unpublished studies mentioned earlier are published in detail, they, together with the earlier studies already mentioned, will provide an insight into the difficulties in assembling controls which should illuminate a good deal of future genetic investigation. Jacobsen was probably led to overemphasize familial factors in carcinoma of the breast because of under-reporting on the part of his control material. More recent studies, having gotten over this hurdle, are now facing a second, more statistical hurdle. Incidentally, in view of the recrudescence of interest in the possible viral etiology of various human malignancies (Gross, 1954), it should be pointed out that even if a virus were implicated in the causation of breast cancer, the observations of the above quoted studies would be in no way invalidated; the geneticist will be in trouble only if he prematurely places a precise genetic interpretation on a finding indicative only of familial factors.

4. FAILURE TO BEAR IN MIND THE UNIQUENESS OF MAN

Finally, there is need to mention the desirability of constantly bearing in mind the uniqueness of man. I must confess, as one who cut his genetical milk teeth on Drosophila, that there are times when I have fallen into the obvious trap of regarding man as an overgrown fruit fly. Others with different backgrounds perhaps see him as an enormous mouse. That this is not the case seems self-evident, and yet I wonder to what extent various of you here have shared this same pitfall with me. That we have learned a great deal from Drosophila and the mouse, there is no doubt, but perhaps the time has come for the flow of knowledge to be more of a two-way proposition.

Man is set apart from all other animals by the accumulation and transmission of a body of knowledge usually termed his culture, that is, by a cultural inheritance no less complex than his biological inheritance. In this culture, man is for what seem to be understandable reasons the pivotal figure, in consequence of which the available information concerning the human species far outstrips our knowledge of any other animal. The relationship which man's culture bears to our efforts to understand him has been explored in detail by many scholars (cf. Huxley, 1941; Simpson, 1949; White, 1949; Kroeber, 1952; Etkin, 1954). There is scarcely a single aspect of our attempts to study man which remains untouched by cultural factors. The uniqueness of man as an object of genetic study has been repeatedly impressed upon our group at Michi-

gan in connection with two of our major interests, namely, the study of human mutation rates, and the detection of genetic carriers of inherited disease. Scarcely a year passes but what some new facet of the differences between man and other animals as subjects for such investigation comes to light.

Consider, first, the study of mutation rates, a subject which Dr. Schull and I have recently reviewed in some detail elsewhere (Neel and Schull, 1954). It has been frequently pointed out that because of the impossibility of controlled matings in human societies, in most mutation rate studies on human material we cannot be sure that a given phenotype attributed to germinal mutation is not either a phenocopy, the result of somatic mutation, or due to complex recombination phenomena. The study on the rate of mutation of the gene responsible for retinoblastoma which Falls and I (1951) carried out some years ago may provide a good example of how this bias operates. Although recognizing the various alternative possibilities just mentioned, in the absence of actual data on the point, we were forced to assume for purposes of calculation that all sporadic cases of retinoblastoma were due to germinal mutation. Now, however, Nachtsheim (1954), in a paper prepared for the current World Population Conference, on the basis of data recently collected on the children of surviving, sporadic cases of retinoblastoma, raises the possibility that our estimate may be too high by as much as a factor of 4 because of the inclusion of phenocopies or cases due to somatic mutation in the mutation rate estimate. I very much doubt that the bias is as great as that, since the bilateral cases, which are most apt to be due to germinal mutation, have a lesser chance of survival than the unilateral, and, if they survive, a lesser change of reproduction. Nevertheless, here may be an excellent example of how this set of biases operates.

Furthermore, even if the phenotype under study is definitely due to germinal mutation, we cannot be sure that it does not result from change at any one of several different loci. Thus, even so apparently specific a disease as classical, sex-linked hemophilia is now a complex of at least two diseases, true hemophilia and plasma thromboplastin component (PTC) deficiency or Christmas disease, a fact that future mutation rate studies on this locus must bear in mind (cf. Symposium: What is Hemophilia? Blood 9: 244–293). It is obviously very unlikely that if some human mutation rate studies involve such compound loci as bithorax, Star, lozenge, vermilion, or white in Drosophila, we will ever be able to dissect the estimates down to their component parts. These facts will result in spuriously high estimates of human mutation rates.

But is it generally appreciated that there may be just as important biases operative in the other direction as regards the detection of mutation resulting in "visibles" (as opposed to lethals) in Drosophila? One of these is the small size of the organism, and the probability of overlooking departures from the norm which on an equivalent scale would be glaringly apparent in man. There is a second factor which has not been adequately stressed in the past. Most estimates of the frequency of mutation resulting in "visibles" in Drosophila are based upon the detection of single individuals showing the effects of sex-linked, recessive mutation, or sex-linked and autosomal dominant mutation. Some of the more refined estimates involve the search in suitably heterozygous strains for animals exhibiting the effects of autosomal recessive mutation. Save where gonadal mosaicism is involved, mutation of this type results in a single affected

individual. Now, many studies have demonstrated the impaired viability of most of the visible mutants of Drosophila. These demonstrations are usually based on departures from an expected 3:1 or 1:1 ratio. Viability may be even more impaired, relatively speaking, when the mutant is one among a culture of normal brothers and sisters. One can argue, then, that under the usual Drosophila culture conditions negative selection probably eliminates a very sizable fraction of mutant individuals before they ever come to the investigator's attention. So far as I am aware, no Drosophilist has ever performed the very simple experiment of introducing into scattered members of a series of wild type cultures of Drosophila, just as the flies are beginning to emerge, a single mutant fly, and seeing how many of these mutants are recovered by an unsuspecting but competent observer three days later. Contrast the situation in human populations, where the physician from the moment of birth is in constant attendance upon the ill and unusual.

Let us deal with equal brevity with the detection of genetic carriers in man (reviews in Neel, 1947, 1948; Falls, 1953; Franceschetti and Klein, 1954). In the final analysis, this is in large part the study of early, manifold, or subclinical gene effects. It has frequently been pointed out that such studies in man are complicated by our inability to standardize the genetic background. It is not commonly realized to what extent this is offset, again, by our extensive background information on man. A single recent example will make the point. Cystic fibrosis of the pancreas is a recessively inherited disease well known to the geneticist. Several years ago, during an unusually hot spell in New York City, it was first recognized that children with this disease are unusually prone to heat prostration (Kessler and Andersen, 1951). Later studies revealed an increased chloride loss in the sweat of such children, the proneness to heat prostration apparently being a consequence of sodium cloride depletion due to a congenital defect in the sweat glands (Darling, et al., 1953). Of even greater interest in the present context is the fact that among 60 close relatives, apparently for the most part parents and siblings, of children with the disease, distributed aong 18 families, 14 were found to have abnormally large amounts of electrolytes in their sweat (di Sant'Agnese, et al., 1954). These presumably are heterozygotes for the gene. From the proportion found to be abnormal, it does not appear that all heterozygotes exhibit the abnormality, but further studies, with more refined techniques, may reveal that the proportion of affected heterozygotes is higher than it appears to be at the present time. Here, then, is a new carrier state. Its recognition is the outgrowth of good clinical medicine plus an awareness of genetic principles. Let us assume a similar disease existed in some laboratory animal. I wonder how rapidly, granting that a susceptibility to heat prostration was recognized, its mechanism could be analyzed. The fact is that there is available to those of us interested in human genetics a richness of ancillary biological techniques which is available for no other living organism.

CONCLUDING REMARKS

Today geneticists are operating on a broad front indeed. At this very moment some members of this Society are participating in Section B-10 at the World Population Conference in Rome. Earlier in the year under UNESCO sponsorship a group which included geneticists met in Paris to try to come to grips with the implications

of differential fertility for the intelligence of future generations. Next month the annual Teaching Institute of the Association of American Medical Colleges will be devoted to the subjects of pathology, microbiology, and genetics. This is undoubtedly the most significant opportunity which has yet developed to present the place of genetics in the modern scheme of medical education. The burden of this presentation will again fall on members of this Society. Less specific but nonetheless tangible evidence of the breadth of our horizons comes from the still active controversy surrounding the subject of heredity in Russia, and the recent Papal statement devoted to the same subject.

As our external relations with other disciplines multiply, we will be tested on an ever-broadening scale. Mindful of the set-backs the study of human heredity has suffered in the past because of an uncritical approach to problems as profound as any with which man has ever grappled, it is imperative that in our present sturdy growth we constantly bear in mind these lessons. All of the pitfalls I have touched upon this evening have been adequately discussed by others in the past. They will bear reiteration in the future. May I suggest, as the "stop, look, and listen" of our discipline, the following four questions: Is my material as homogeneous as it can be made in the light of present knowledge? Have I considered exhaustively the various genetic hypotheses applicable to the situation under study? Where controls are indicated, have they been assembled by the best sampling methods available? And finally, have I been led to specious or unwarranted conclusions by thinking in terms of non-human material?

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